

Myocarditis is a severe disease, which affects 1,5 million people per year. It is estimated that every tenth patient will develop heart failure. During the first, acute phase, disease is caused mostly by infectious agents (viruses, bacteria, protozoa, fungi), but also physical and chemical factors. In some patients, it progresses into chronic myocarditis, where autoimmune mechanisms play a major role. During chronic myocarditis, patients' own immune cells activity will remain a damaging factor. Then, it leads to pathological remodeling of inflamed parts of heart muscle and often development of inflammatory dilated cardiomyopathy.

Exact molecular mechanisms leading to cardiomyocyte (cell of cardiac muscle) remodeling have not been understood. It is postulated that this process depends on humoral immunity (associated with antibodies) or cell immunity (destroying other cells by activating "death" pathways).

**The aim of this project is to assess the impact of selected cytokines (molecules secreted by immune cells) on cardiomyocytes.** Cytokines are a group of molecules with multivariate impact on different types of cells. Animal studies and clinical experience showed the potential relationship of some cytokines with the course of myocarditis. In this study we selected six cytokines with probably the highest significance in pathogenesis of myocarditis.

The study is comprised of two parts: (1) cell culture and (2) tests of myocardial biopsy samples from chronic myocarditis or inflammatory dilated cardiomyopathy subjects. Cell culture experiments will be conducted using induced pluripotent stem cells (iPSC) derived cardiomyocytes. iPSC are almost identical to human embryonic stem cells, but are obtained by reprogramming adult human cells (usually fibroblasts). Cardiomyocytes are obtained by treating iPSC with a mixture of inhibitors and inductors of signaling pathways at appropriate time intervals. After 14-20 days spontaneously beating cells with cardiomyocyte phenotype are obtained. These cardiomyocytes pose advanced laboratory model of cardiac cells.

Differentiated cells will be then treated with selected cytokines. Impact of these cytokines will be assessed with modern imaging techniques (confocal and holographic microscopy), metabolic profiling techniques and flow cytometry. They will determine how cytokines impact cardiomyocyte morphology, metabolism, and viability. Additionally, gene expression will be assessed in impacted cells. Subsequently, the most affected genes will be identified through computer analysis. Then, the expression of previously identified and the most affected genes, will be assessed in myocardial biopsy samples. It will allow to evaluate the correlation of laboratory observations with clinical results. Furthermore, the connection between the level of these genes' expression and clinical outcomes will be assessed.

The study will provide a unique opportunity to better understand the underlying mechanisms of myocarditis and to develop novel diagnostic and treatment possibilities.